A New Biological Approach to Vital Pulp Therapy

Bruce Rutherford
Mark Fitzgerald
University of Michigan School of Dentistry, 1011 N. University, Ann Arbor, MI 48109-1078

ABSTRACT: Molecular biology is providing opportunities to develop new strategies or agents for the treatment of a wide variety of diseases. The availability of large amounts of highly purified proteins produced by recombinant DNA techniques is an obvious example. Recent evidence has implicated proteins belonging to the bone morphogenetic protein (BMP) subgroup of the transforming growth factor beta supergene family in tooth formation and dentinogenesis. It has long been known that bone and dentin contain bone morphogenetic protein activity. Recently, recombinant human BMP-2, -4, and -7 (also known as OP-1) have been shown to induce reparative dentin formation in experimental models of large direct pulp exposures in permanent teeth. The manner in which these agents act appears unique. New reparative dentin replaces the stimulating agents applied directly to the partially amputated pulp. Hence, the new tissue forms contiguous with, largely superficial to, and not at the expense of the remaining vital pulp tissue. This suggests a therapeutic approach permitting the induction of a predetermined and controlled amount of reparative dentin. Additionally, OP-1 has been associated with the formation of reparative dentin after application to a freshly cut but intact layer of dentin. These findings may provide future clinicians with additional options for the treatment of substantially damaged or diseased vital teeth.

Key words. Bone morphogenetic proteins, reparative dentin, reactionary dentin, odontoblast differentiation.

(I) *Introduction*

Vital pulp therapy may be broadly defined as any aspect of restorative dental treatment intended to minimize trauma to the dental pulp. More specifically, it may refer to those procedures intended to protect the pulp, in the absence of exposure, from phlogistic or toxic effects of dental materials, or microbiological, thermal, and mechanical damage. These procedures typically involve the use of cavity liners and bases. Vital pulp therapy may also involve the transdentinal (indirect) and direct stimulation of tertiary dentin matrix formation in and by vital dental pulps. Materials or agents used to stimulate the formation of such tertiary dentin matrices may be categorized as non-biologic (physical, chemical, etc.) or biologic, based upon the nature of the therapeutic material. Here, we focus on biologic agents—compounds synthesized in and by biological systems. Calcium hydroxide, cyanoacrylates, dental cements such as zinc oxide or phosphate preparations, and similar materials are, under this definition, non-biologic and therefore are not reviewed. Reviews and textbook chapters covering such agents are available; for example, see Glass and Zander (1949), Hess (1950), Haskell *et al.* (1978), Seltzer (1988), Stanley (1989), and the references therein. Most studies in this area have dealt with the preservation of pulp vitality and stimulation of tertiary dentin matrices as coupled biological phenomena. The repair or regeneration of pulp tissue is usually considered in combination with dentinogenesis and not as a therapeutic goal unto itself. This is a logical extension of the concept of the dentin-pulp complex as an integral structural and functional unit. Therefore, much of the current data is interpreted from this perspective. Success for most experiments is defined in the context of formation of new tertiary dentin matrices. Materials such as hard-set calcium hydroxide are described as giving rise to dentin formation within or at the expense of the pulp tissue. Hence, such reparative dentin is described as reducing the size of the pulp chamber deep to the remaining layer of the therapeutic material. Evidence from recent experiments using recombinant human proteins in direct pulp exposures suggests that a new paradigm may soon emerge (data reviewed below). In this paradigm, the therapeutic agent induces its own replacement with a fibrous connective tissue reminiscent of immature pulp that subsequently mineralizes, leaving a mass of reparative dentin *in situ*. This reparative dentin forms superficial to and not at the expense of the pulp tissue.

Confusion exists regarding the categorization of tertiary dentin matrices. The term "osteodentin", a form of

dentin found in primitive fish (Orvig, 1967), is often applied to mammalian tertiary dentin, which, due primarily to the presence of cells surrounded by mineralized matrix, superficially resembles bone at the level of resolution of the light microscope. When referring to tertiary dentin matrices of mammalian teeth, we prefer to use the terms "reparative" or "reactionary" dentin (Lesot *et al.*, 1993). By these definitions, the distinction between reparative and reactionary dentin is based upon the origin of the odontoblasts responsible for its production. Reparative dentin is a tertiary dentin matrix formed by new odontoblast-like cells in response to a specific stimulus, while reactionary dentin is formed by surviving odontoblasts subjacent to diseased or otherwise damaged dentin. This focus on the nature and activity of the cells responsible for the production of the tertiary dentin matrix is both theoretically and practically useful. Different biologically based strategies for the therapeutic induction of each type of tertiary dentin may necessarily be predicated upon the nature of available cells capable of responding to the initial stimulus.

An excellent review of dentinogenesis has recently been published (Linde and Goldberg, 1993). Dentinogenesis in adult teeth following loss of odontoblasts or the partial amputation of pulp tissue has been reviewed (Baume, 1980). Lesot and colleagues (Lesot *et al.*, 1993) reviewed recent experimental approaches to the induction of odontoblast differentiation with regard to reparative and reactionary dentinogenesis. Information pertaining to biologically based approaches to vital pulp therapy intersects at least partially with that pertaining to odontoblast differentiation. Therefore, we attempt to build upon this subject by reviewing relevant new data from the perspective of whether the clinical access to the vital pulp is direct or transdentinal. The search for biological dentinogenic molecules has most recently focused on bone and dentin matrices. Literature published and indexed as of February, 1995, has been surveyed.

(II) *Biological Dentinogenic Molecules: Non-diffusible Extracellular Matrix Proteins*

Collagen

The rationale for the use of collagen to induce bone or dentin formation was provided by evidence documenting that biological mineral formation initiates within collagen fibers and conjecture that collagen gels may provide an appropriate scaffolding for tissue formation. In this regard, native collagen gels were not as efficacious as calcium hydroxide in promoting pulp repair and reparative dentin formation in rats (Johannson *et al.*, 1963). Similar results were observed in dog pulp exposures treated with xenogenic, acid-soluble, collagen-enriched solutions which had been enzymatically treated to reduce antigenicity (Carmichael *et al.*, 1974), xenogenic collagen sponges, or other types of collagen preparations (Dick and Carmichael, 1980).

Substantial formation of pulp without reparative dentin formed in 30 days in experimental dog pulpal wounds treated with direct application of an enriched, acid-soluble autologous skin collagen solution (Bimstein and Shoshan, 1981). A similar preparation of monkey skin collagen was associated with pulpal healing 60 days after direct application to exposed pulps in pulpotomized monkey teeth. Dentin bridges were evident in some anterior teeth. Pulp tissue was present superficial to zones of reparative dentin, suggesting that this collagen solution induced the formation of a pulp-like tissue and that further mineralization of the new tissue might occur. Since only one healing interval was evaluated, no evidence regarding the initial development or fate of this tissue is available (Fuks *et al.*, 1984). In a follow-up study, a commercial preparation of collagen was associated with pulpal inflammation and necrosis (Fuks *et al.*, 1991). These studies suggest that naturally sourced collagen, alone, is currently not a promising material for biological approaches to vital pulp therapy. Since no further studies are available, many questions regarding the efficacy and safety of collagen preparations remain unanswered.

Fibronectin

Another approach to therapeutic dentinogenesis in direct pulp exposures is to provide an organizing surface or substrate in substitution for the basement membrane. Dental papilla cells attach to a basement membrane prior to terminal differentiation into odontoblasts (Thesleff and Hurmerinta, 1981; Kollar, 1983; Ruch, 1985). Cell attachment factors such as fibronectin were suggested (Veis, 1985). Fibronectin or type I collagen *in vitro* failed to induce odontoblast elongation and polarization (Lesot *et al.*, 1985), but in combination with transforming growth factor β_1 (TGF-β_1) was associated with the initiation of odontoblast differentiation in dental papilla cell cultures (Lesot *et al.*, 1993). Atubular and tubular dentin formation occurred on bovine plasma fibronectin-coated Millipore filters four weeks after implantation of the filters in dog pulps (Tziafas *et al.*, 1992a). Whereas these data suggest that xenogenic fibronectin may be associated with induction of reparative dentinogenesis, an atubular matrix formed in contact with the membrane while tubular dentin appeared limited to the surface of the newly formed atubular matrix. The authors suggest that the absorption of autologous molecules to the initial matrix may be a factor influencing the subsequent pattern of mineralized matrix formation. A pattern of tertiary matrix formation where tubular dentin forms on the surface of atubular dentin has been repeated in other models of reparative dentinogenesis (Nakashima, 1990,

1994; Tziafas and Kolokuris, 1990; Rutherford *et al.*, 1993, 1994), discussed below.

Diffusible extracellular matrix molecules: growth factors

Demineralized dentin powder induces ectopic bone formation (Bang and Urist, 1967a,b; Yeomans and Urist, 1967; Huggins and Urist, 1970; Urist, 1971; Bang, 1972, 1973; Bang and Johannessen, 1972; Bang *et al.*, 1972; Inoue *et al.*, 1986). These studies were stimulated by Marshall Urist's seminal demonstration of the *in vivo* ectopic osteogenic activity of demineralized bone powder (Urist, 1965). The subsequent search for the molecules in bone responsible for osteogenic activity led to the cloning of genes coding for some of the osteogenic (bone morphogenetic) proteins (Wozney *et al.*, 1988; Ozkaynak *et al.*, 1990, 1992). Bone (Hauschka *et al.*, 1986) and dentin (Finkleman *et al.*, 1990) matrices also contain several other biologically active molecules.

Osteogenic (OP) or bone morphogenetic (BMP) proteins comprise a subgroup of a larger family of structurally related proteins, designated the TGF-β superfamily, and are implicated in diverse biological activities involving differentiation, tissue morphogenesis, regeneration, and repair. Eight members of the BMP subfamily have been identified: BMP-2, -3, -4, -5, -6 (Wozney *et al.*, 1988; Celeste *et al.*, 1990), and OP-1, -2, and -3 (Ozkaynak *et al.*, 1990, 1992). OP-1 and -2 are also known as BMP-7 and -8. Like other members of the TGF-β family of proteins, the BMPs are synthesized and secreted as large precursors from which a large peptide (pro-domain) is subsequently cleaved to form the mature carboxy-terminal, dimeric, signaling molecule (Massagué, 1990). No biological functions have been assigned to the large monomeric pro-domain, which is thought to be cleaved during or immediately after secretion by cells *in vitro* (Fig.). Recombinant BMP-2, -4, -5 (Wozney, 1992), and OP-1 (Sampath and Rueger, 1994) induce intra- and extraskeletal bone formation.

Dentin fragments introduced into pulp during mechanical exposure or implanted demineralized dentin induces mineralized tissue formation (Anneroth and Bang, 1972; Seltzer and Bender, 1984). The dentin-associated activity can be partially removed by extraction with 4 M guanidine-HCL, suggesting that dentin matrices possess an extractable dentinogenic activity (Nakashima 1989). Extracellular matrix (ECM) deposition onto the implanted demineralized dentin surface preceded morphological evidence of cell differentiation toward odontoblast-like cells.

Autologous demineralized dentin chips experimentally implanted into dog dental pulps were covered with, first, a layer of atubular matrix, followed by a layer of tubular-like predentin and associated odontoblast-like cells (Tziafas and Kolokuris, 1990). In no case was tubular-like dentin deposited directly onto the implanted chips. In a short-term follow-up study, the effects of autologous demineralizd and non-demineralized dentin chips implanted into dog pulps (Tziafas *et al.*, 1992b) were compared. The non-demineralized chips were prepared to possess both dentin and predentin surfaces. No inactive control materials were compared. Only the predentin surface was associated close to new odontoblast-like cells, while demineralized or non-demineralized dentin was associated with the formation of new extracellular matrix before the appearance of odontoblast-like cells occurred. Attachment proteins, such as fibronectin, adhering to the dentin matrix were suggested to mediate cell differentiation; however, the data do not support such speculation.

These studies are interpreted to suggest that dentin is capable of inducing the production of tertiary dentin-like matrices when implanted into dental pulps. No quantitation or statistical analyses are included. It would be of interest to know: (1) the proportions of successes and failures, (2) the relative potency of denaturant extracted and non-extracted demineralized dentin, and (3) an estimate of the variability of the tissue response to the several preparations.

The observations that non-collagenous proteins with bone morphogenetic protein (BMP)-like activity could be isolated from dentin matrix (Butler *et al.*, 1977; Conover and Urist, 1982; Mera, 1988; Bessho *et al.*, 1990) indicated that at least some members of the BMP family of proteins may be involved in tooth development and dentinogenesis. Similar suggestions have been made for TGF-β and IGF-I and -II (SGF-II), since these molecules, present in bone extracellular matrix, have also been detected in adult dentin (Finkleman *et al.*, 1990). However, dentin extracts with BMP activity have not been further characterized. Additional data suggesting that IGF-I, TGF-$\beta_{1,2,3}$, and some of the BMPs (Table) are involved in tooth development were initially suggested by immunolocalization or *in situ* hybridization analyses of embryonic tooth development (Heine *et al.*, 1987; Lehnert and Akhurst, 1988; Lyons *et al.*, 1990; Pelton *et al.*, 1990; Joseph *et al.*, 1993).

TGF-$\beta_{1\text{-}3}$ mRNA and proteins are expressed at various stages in developing teeth (Heine *et al.*, 1987; Lehnert and Akhurst, 1988; Pelton *et al.*, 1990; Vaahtokari *et al.*, 1991; Jepsen *et al.*, 1992; Heikinheimo *et al.*, 1993; Bègue-Kirn *et al.*, 1994). One important observation from these studies is that frequently m-RNA and protein are not co-expressed. This differential expression may reveal clues regarding the mechanisms whereby a growth factor or morphogen influences tissue formation (*e.g.*, autocrine *vs.* paracrine). Protein association with a tissue distinct from that synthesizing the m-RNA could reflect the distribution of receptors for that protein or other mechanisms for tissue-specific distribution of soluble

factors. However, normal tooth development and eruption in a TGF-β_1 knockout (TGF-β_1^-/TGF-β_1^-) mouse (D'Souza *et al.*, 1995) suggest that fetal expression of this gene is not essential for normal tooth development. It is possible that maternal proteins or other fetal genes provide the necessary functions.

In the mouse, BMP-2 mRNA is expressed first in the inner enamel epithelium, then (E16) in dental mesenchyme and later in odontoblasts during late bell stage (Lyons *et al.*, 1990; Vainio *et al.*, 1993). BMP-2 mRNA in human fetal tooth buds, detected by the reverse transcriptase-polymerase chain reaction, was temporally correlated with odontoblasts only after formation of primary dentin matrix and not earlier during cap and bell stages (Heikinheimo, 1994). In mice, the pattern of BMP-4 expression differs both temporally and spatially from that of BMP-2 (Vainio *et al.*, 1993). BMP-4 mRNA expression is first detected in association with the initial epithelial thickenings marking that epithelium which subsequently invaginates into subjacent mesenchyme. Later, it is transiently expressed in both the epithelium and the condensing mesenchyme, where expression is limited to those areas occupied by pre-odontoblasts. Transient expression is then associated with the enamel epithelium prior to ameloblast differentiation. BMP-4 expression was related to the pattern of reciprocal epithelial-mesenchymal interactions (EMI) proposed to mediate inductive interactions (Vainio *et al.*, 1993) which appear to function in tooth formation (Kollar, 1983). BMP-4 induces the expression of the homeobox-related genes Msx-1 and Msx-2 (Vainio *et al.*, 1993) which are expressed during (MacKenzie *et al.*, 1991, 1992; Jowett *et al.*, 1993) and may function in tooth development (Satokata and Maas, 1994). In contrast, BMP-4 was not detected in an earlier study of mouse tooth development (Jones *et al.*, 1991), and BMP-4 mRNA expression in developing human tooth buds was restricted to the dental papilla throughout cap and bell stages, while osteoblasts of the surrounding alveolar bone continuously expressed BMP-2 and -4 mRNA (Heikinheimo, 1994).

In human tooth buds, BMP-6 protein was immunolocalized to the enamel epithelium during cap-stage shifting to the dental papilla during the bell stages and was predominantly but not exclusively expressed by odontoblasts at the time of mineralization. This pattern of expression is proposed as consistent with a role in EMI (Heikinheimo, 1994). Similarly, OP-1 has been immunolocalized to the basement membrane of the dental lamina and later, as the dentin matrix forms, to odontoblasts (Valecillos *et al.*, 1993). Given the high degree (70-80%) of sequence identity between BMP-6 and OP-1 (Celeste *et al.*, 1990), it will be necessary to rule out cross-reactivity between the anti-OP-1 antibody and BMP-6 to confirm OP-1 expression. The anti-BMP-6 antisera used in these studies failed to detect OP-1 (BMP-7) produced *in vitro* Wall *et al.*, 1993).

BMP-6 immunolocalization appeared to be limited to the cytoplasm of the immuno-positive cells (Heikinheimo, 1994). The anti-BMP-6 antibody used in this study was raised against the pro-domain peptide (Fig.) of the molecule to limit the potential for cross-reactivity to BMP-5 and OP-1, since this domain has the lowest level of sequence identity (Celeste *et al.*, 1990). In studies of BMP-6 protein expression, the data suggest that the predominant form of the molecule immunodetected in steady-state protein *in vivo* is the pro-domain of the cleaved molecule. The antibody immunoprecipitated the cleaved signal protein (Fig.), but it failed to detect this form in Western blots (Wall *et al.*, 1993). The presence of detectable amounts of immunoreactive BMP-6 polypeptide in the cytoplasm of odontoblasts, papilla cells, osteoblasts, and pre-ameloblasts (Heikinheimo, 1994) suggests that the pro-domain accumulates intracellularly. This could mean that the cleaved pro-domain (Fig.) functions intracellularly independently of the remainder of the molecule. Absence of a clear demonstration of the signaling form (Fig.) of BMP-6 in these studies weakens the case for a role of BMP-6 in EMI dur-

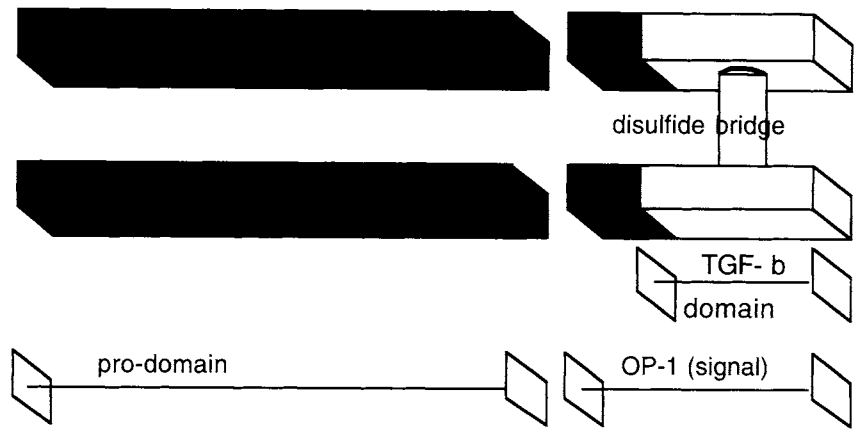

Figure. Osteogenic protein-1 (BMP-7). Schematic diagram of OP-1. The prodomain is cleaved during or just after secretion by cells *in vitro*. The extent of sequence identity among the various BMP proteins is less in this domain than in the cleaved signaling molecule which contains the canonical TGF-β domain. The signaling molecule is associated with the known biologic activities.

ing tooth formation.

Further evidence of a role of IGF-I, TGF-β, BMP-2, and a non-collagenous extract from dentin matrix (which may contain these as well as other molecules (Finkleman *et al.*, 1990) in tooth development has been published (Bègue-Kirn *et al.*, 1992, 1994). These studies revealed that a non-collagenous dentin extract (Smith *et al.*, 1990) induced odontoblast differentiation in mouse papilla cultures. This activity was inhibited by pre-incubation with anti-TGF-β_1 and anti-TGF-β_2 antibodies, suggesting that growth factors known to exist in dentin matrix could induce odontoblast differentiation (Bègue-Kirn *et al.*, 1992). Subsequently, these investigators have shown that combinations of TGF-β_1 and heparin, IGF-I and BMP-2, and IGF-I and TGF-β_1 mimicked the effect of the dentin matrix extract on odontoblast differentiation *in vitro*. However, different patterns of gene expression were detected in odontoblasts developing *in vitro* and *in vivo* (Bègue-Kirn *et al.*, 1994). Data regarding expression of TGF-β superfamily genes in developing teeth are summarized in the Table.

Cultured human adult pulp cells derived from a single human adult pulp were recently reported to contain BMP-2, -4, and -6 mRNA (Takeda *et al.*, 1994). Immortalized mouse papilla cells express BMP-2, -4, and OP-1 mRNA (Mary MacDougal, personal communication). We have cloned cDNAs for BMP-2, -4, and OP-1 from RNA prepared from human pulps and human pulp cells in culture by the reverse transcriptase-polymerase chain reaction. The primers, unique to each gene, defined a region which spanned known exon splice sites, ruling out amplification of genomic DNA which may contaminate total cell RNA preparations. Identity of the cDNAs was confirmed by sequencing (R.B. Rutherford, unpublished observations). Hence, it appears that culturing pulp cells does not alter expression of these genes. Given the heterogenous cell populations which comprise the dental pulp and the fibroblastic appearance of those cells which grow from explants, it will be of interest to determine which pulp cells express these genes and to study the regulation of expression. It will also be important to determine which (if any) cells of adult pulps produce each protein, because BMP mRNA expression is not perfectly correlated with the detection of protein (Wall *et al.*, 1993).

These data suggest that several members of the TGF-β supergene family (TGF-$\beta_{1,2,3}$, BMP-2, -4, -6, and OP-1) and IGF-I may play multiple roles during tooth development. These roles could include mediating epithelial-mesenchymal interactions, odontoblast differentiation, and dentinogenesis and facilitating tooth eruption. TGF-β and IGF-I and -II (Finkleman *et al.*, 1990) as well as a molecule(s) with BMP activity are present in dentin. Low levels of at least some of these mRNAs are present in adult pulps, suggesting that the proteins may be inducible or constitutively synthesized. Finkleman and co-workers speculate that caries may release biologically active molecules from dentin which stimulate the formation of reactionary or reparative dentin (Finkleman *et al.*, 1990). It will be interesting to determine the effects of these molecules on odontoblast differentiation, gene expression, protein synthesis, and, ultimately, the production of a mineralized dentin matrix.

Most of the evidence implicating growth factors in tooth development is circumstantial and incomplete. In addition, great care must be exercised in the interpretation of studies utilizing *in situ* hybridization which fail to utilize control sense as well as antisense riboprobes. Interpretation of the specificity of the proposed patterns of expression is more difficult without such control data. Because substantial regions of the BMP nucleic acid and protein sequences are identical or nearly so (Fig.), the regions of the molecules from which cDNAs for riboprobe preparation are derived must be carefully selected to minimize cross-hybridization. Riboprobes prepared by reverse transcription of specific cDNAs are routinely hydrolyzed to approximately 200 bp before *in situ* hybridization. This may increase the chances of cross-hybridization. Similarly, the extent to which antibodies to the different proteins cross-react must be determined before definitive claims implicating a specific gene product may be made. It is also important to note that expression of an mRNA does not necessarily establish that physiologically significant amounts of the protein are produced (Wall *et al.*, 1993).

(III) *Reparative Dentinogenesis—Direct Approach*

The data reviewed above have inspired and stimulated current efforts to study biological approaches to the preservation of pulp vitality and direct stimulation of reparative dentin formation. Most recently, this work has focused on BMP-2 and -4, OP-1, TGF-β, and platelet-derived growth factor (PDGF). The first study demonstrated that a solubilized, unpurified preparation from allogenic dentin which possessed BMP activity stimulated reparative dentinogenesis in partially amputated pulps of adult dog teeth (Nakashima, 1990). The replacement of the implant with a loose fibrous connective tissue and a small amount of an atubular tertiary matrix with cellular inclusions occurred by one month. By two months, approximately half of this tissue was replaced with atubular and associated tubular-like reparative dentin. Very little reparative dentin formed in the control albumin-treated teeth. A qualitatively similar result from use of a crude xenogenic bone-derived BMP preparation was subsequently described (Lianjia *et al.*, 1993). The crude dentin or bone extracts with BMP activity could contain other active factors (Finkleman *et al.*, 1990; Smith *et al.*, 1990), confounding identification of dentinogenic

agents.

Smith and co-workers described the capacity of a crude dentin preparation extracted by EDTA and collagenase digestion to stimulate reparative dentinogenesis in small pulp exposures in ferrets (Smith *et al.*, 1990). The active factor(s) was not identified, but the capacity of this dentin extract to influence odontoblast differentiation *in vitro* was inhibited by anti-TGF-β_1 and anti-TGF-β_2 antisera as described above (Bègue-Kirn *et al.*, 1992).

In a follow-up study to her previous work, Nakashima reported that recombinant human BMP-2 and -4, but not TGF-β, induce reparative dentinogenesis in dogs when placed on partially amputated dental pulps (Nakashima, 1994). Similar data from non-human primate studies using recombinant human OP-1 have been reported (Rutherford *et al.*, 1993, 1994). The selection of recombinant human OP-1 for use in these dentinogenesis experiments was predicated upon data derived from experiments with bone, since the specific protein(s) responsible for the BMP activity in dentin has not been identified. OP-1 and, to a lesser extent, BMP-2 were shown to account for most of the extractable BMP activity (Sampath *et al.*, 1990). In addition, OP-1 (BMP-7) may be substantially more potent than BMP-2 (Wang *et al.*, 1990). The OP-1 studies demonstrate that:

(1) OP-1, but not PDGF, complexed with an insoluble collagen delivery vehicle (CM) predictably and reliably induces reparative dentinogenesis in non-human primates;

(2) the initial response appeared to be a fibroblastic and angiogenic invasion of the OP-1/CM, resulting in a mineralizing mass of new pulp tissue which forms largely superficial to and not at the expense of existing pulp tissue;

(3) the amount of reparative dentin formed was proportional to the total mass of OP-1/CM placed on the freshly amputated pulp;

(4) OP-1/CM reparative dentin formation appeared to be independent of the amount of coronal or radicular pulp removed;

(5) mineralized matrix constituted 95% of the reparative dentin by six months in monkeys; and

(6) OP-1/CM-induced reparative dentin is initially predominantly atubular, with odontoblast-like cells associated with areas of tubular-like dentin appearing by one month.

Type I collagen from bovine bone was used as the carrier of recombinant human OP-1 in the primate model of reparative dentin formation (Rutherford *et al.*, 1993, 1994). This carrier is bone powder which has been demineralized, sieved, extensively extracted with quanidine hydrochloride, washed in water, and lyophilized (Sampath and Reddi, 1981). In contrast, Nakashima used dog dentin prepared similarly and mixed with chondroitin 6-sulfate and acid-soluble rat tail tendon collagen (Nakashima, 1994). Addition of recombinant BMP-2 and -4 to this dentin-derived collagen approximately doubled (82% *vs.* 42%) the amount of reparative dentin formed during a 60-day healing period in dog permanent teeth (Nakashima, 1994). In contrast, the bovine-derived collagen carrier was uniformly, totally inactive with regard to reparative dentin formation in primates over the six-month healing interval (Rutherford *et al.*, 1993, 1994). Approximately 30% of all carrier-treated teeth had at least partially vital but inflamed pulps after 30-180 days (R.B. Rutherford, unpublished data). These differences in response to the carrier could be related to differences in the source of the collagen, the methods of preparation (Nakashima, 1989; Sampath *et al.*, 1990), the size of the defect, or the animal model (dogs *vs.* monkeys).

These data demonstrate that reparative dentin forms in response to recombinant BMP-2 and -4 and OP-1. The tissue initially appears atubular, with cellular and soft-tissue inclusions. Subsequently, a more tubular form of matrix with associated odontoblast-like cells appears attached to the mass of atubular matrix. This is similar to the pattern of tissue formation occurring in response to implanted dentin chips (Tziafas and Kolokuris, 1990; Tziafas *et al.*, 1992b) and fibronectin-coated filters (Tziafas *et al.*, 1992b). This observation supports the idea that some extracellular structure is a prerequisite to odontoblast differentiation and tubular dentin formation (Kollar, 1983; Veis, 1985) and implicates atubular dentin in that role.

FUTURE DIRECTIONS

The animal-efficacy or proof-of-principle experiments described (Nakashima, 1990, 1994; Rutherford *et al.*, 1993, 1994) above raise several interesting questions and suggest further experiments in the area of biologic approaches to vital pulp therapy. All of these experiments have utilized clinically healthy teeth—that is, no attempt was made to induce pulpitis specifically prior to exposure and partial amputation of the vital pulp. Clearly, it is important to attempt to determine the degree and extent of existing pulpitis that can be successfully treated. Currently, there is no precise definition of "irreversible" pulpitis, nor are there definite clinical or biochemical predictors of the extent of pulpitis or the capacity of an inflamed pulp to recover. Discrepancies exist between the signs and symptoms of pulpitis and the histological evidence of pulpal inflammation (Fitzgerald and Heys, 1991). In the absence of radiographic evidence of periapical periodontitis, often few clues are available to suggest the extent of pulpal pathology. Alternatively, a frankly purulent pulp is generally considered to be an irreversible condition. However, it is possible that vital pulp remains deep to inflamed and

TABLE

TGF-βs and Tooth Development

Gene	Temporospatial Expression of mRNA	Protein
TGF-β_1	(1) early dental epithelium and adjacent mesenchyme; (2) dental papilla; (3) stellate reticulum[1]	in the stellate reticulum from bell stage to just prior to eruption[2]
TGF-β_2	(1) non-condensing mesenchyme lateral to that expressing TGF-β_1; (2) inner enamel epithelium; (3) odontoblast layer[1]	human[1]: (1) dental lamina and enamel organ (2) odontoblasts
TGF-β_3	(1) non-condensing mesenchyme lateral to that expressing TGF-β_1; (2) inner enamel epithelium; (3) odontoblast layer[1]	N/A
BMP-2	mouse: (1) dental epithelium (2) papilla (3) odontoblasts[3] humans: (1) odontoblasts[4] (2) pulp cell cultures[5]	N/A
BMP-4	mouse: (1) early dental lamina (placode)[6] (2) dental epithelium[6] (3) papilla (pre-odontoblast region)[6] (4) inner dental epithelium (prior to ameloblast differentiation)[6] human: pulp cell cultures[5]	N/A
BMP-6	human: pulp cell cultures[5]	human: (1) dental epithelium[4] (2) dental papilla[4] (3) odontoblasts[4]
OP-1	mouse: (1) dental lamina basement membrane[7] (2) odontoblasts[7] human: adult permanent pulp tissue and pulp cell cultures[8]	human: 2° dentin[8]

[1]Heine *et al.*, 1987; Lehnert and Akhurst, 1988; Pelton *et al.*, 1990; Vaahtokari *et al.*, 1991; Heikinheimo *et al.*, 1993; Bègue-Kirn *et al.*, 1994.

[2]D'Souza *et al.*, 1990; Wise and Fan, 1991.

[3]Lyons *et al.*, 1990; Vainio *et al.*, 1993.

[4]Heikinheimo, 1994.

[5]Takeda *et al.*, 1994.

[6]Vainio *et al.*, 1993.

[7]Valecillos *et al.*, 1993.

[8]Rutherford RB (unpublished data).

even abscessed tissue. Theoretically, removal of the diseased tissue to expose the deeper vital pulp and the application of a dentinogenic molecule-therapeutic agent could lead to preservation of a vital and functional tooth. We are currently attempting: (1) to model pulpitis *in vivo*, (2) to develop diagnostic criteria for qualitative and quantitative assessment of pulpitis, (3) to preserve the remaining pulp therapeutically by inducing a protective layer of reparative dentin, and (4) to correlate this outcome to the nature and extent of the inflammation. We are also attempting to determine the efficacy of the OP-1/collagen carrier combination to repair periapical tissues lost through disease or endodontic manipulation. The opportunity to induce controlled amounts of reparative dentin anywhere in the pulp chamber or root canal reinforces the need for the development of direct assays of pulpal inflammation and the determination of predictors of pulpal healing. Clinicians encountering an exposed pulp could then diagnose the extent of disease and accordingly prescribe direct vital pulp therapy, root canal, or extraction.

These studies suggest that one or more of the known BMP molecules can induce reparative dentinogenesis in humans and stimulate current efforts to develop an approach to vital pulp therapy based upon this biological activity. It appears possible to induce the formation of a mass of reparative dentin therapeutically in a uniquely useful way. In contrast to the pulpal response to calcium hydroxide, BMPs stimulate the formation of a mass of reparative dentin largely superficial to and not at the expense of the pulp tissue. This tissue appears to form first as a highly vascular fibrous connective tissue, perhaps resembling imma-

ture dental pulp, that mineralizes over a period of time. The size and shape of the reparative dentin are controllable since it replaces the OP-1/carrier complex (Rutherford *et al.*, 1993)—that is, the reparative dentin may be induced to fill a dentinal defect to a desired size and shape by extending the BMP/carrier complex superficial (coronal) and lateral to the exposure site. The dentinogenic response is dependent upon placing the therapeutic complex on a vital dental pulp but appears to be independent of the coronal extent of the vital tissue, since, in non-human primates, reparative dentin formed when OP-1 was placed at the orifice of the root canals (Rutherford *et al.*, 1994) as well as at the coronal surface of the pulp chamber (Rutherford *et al.*, 1993). By six months, this tissue is comprised of about 95% mineralized matrix, and the pulpal surface is lined mostly by quiescent-appearing cells (Rutherford *et al.*, 1994), suggesting that tissue formation had ceased. If these observations hold true for humans, the reliable therapeutic induction of reparative dentinogenesis and preservation of pulp vitality promise a conservative and more satisfactory physiological clinical endpoint to the treatment of vital yet substantially diseased or damaged dental pulps than is currently available. Currently, such teeth are either treated endodontically or extracted.

Optimum activity for the osteogenic proteins *in vivo* (Wang *et al.*, 1990; Sampath *et al.*, 1992) is achieved by combining them with a carrier molecule and implantation of the combination as a solid mass. In most of the osteogenic protein research over the last decade, bone-derived collagen has been utilized as the delivery matrix. Whereas OP-1 has osteogenic activity when delivered *in vivo* in solution, the use of the collagen-based carrier substantially enhances the activity. As described above, collagen-based carriers were used to deliver BMP-2 and -4 (Nakashima, 1994) and OP-1 (Rutherford *et al.*, 1993, 1994) in animal experiments of reparative dentin formation. However, no data have been published comparing the effects of the collagen-based delivery systems with those of other systems. The OP-1/collagen carrier used in the monkey experiments is a powder which is dampened with sterile saline and delivered with an amalgam carrier. It has a consistency resembling wet snow which has advantages but is probably not adequate for all potential clinical situations. There is a need to develop safe, effective, biocompatible, and biodegradable synthetic carriers with different physical properties such as adhesiveness, structural rigidity, and radiopacity.

It is clear that, at least in two animal models, recombinant human BMPs reliably induce a localized and predetermined amount of reparative dentin. The lack of dentinogenic activity of TGF-β_1 and PDGF-BB suggests that the pulp is restricted in its capacity to respond to morphogens or growth factors. It is possible that there are other genes, related or unrelated to the BMPs, specifically involved in dentin formation. Any clues to the existence of such molecules should be pursued.

Our hypothesis is that adult dental pulps contain cells responsive to at least some BMPs and that the interaction stimulates (either directly or indirectly) dental pulp cells to proliferate, migrate, adhere (attach), and differentiate to form reparative dentin. We subscribe to the dictum that the pathway to understanding mechanisms and developing therapeutics travels through structure. However, no studies exist which comprehensively address the fundamental biological processes leading to reparative dentinogenesis. The cascade of molecular and cellular events central to this event has not been defined. The regulation of odontoblast differentiation is not understood. That a predetermined amount of reparative dentin may be induced by OP-1 is clear. The compositon of this tissue is not known; however, histologically, it appears to be a mix of atubular and tubular dentin. It is important to characterize this tissue and study the long-term viability of irregular or atubular dentin. Atubular dentin may be more caries-resistent, reduce post-operative sensitivity, and provide an improved surface for dentin bonding. Optimal reparative dentin may comprise a superficial layer of atubular dentin with a deeper layer of tubular-like dentin. Understanding regulation of odontoblast differentiation is and should be a major goal of pulp biology. Controlling the differentiation of odontoblasts should lead to the capacity to regulate the type of reparative dentin formed therapeutically, *e.g.*, irregular or tubular dentin. Therefore, from this perspective, as from others, it is important to understand odontoblast differentiation thoroughly, dentin formation specifically, and tissue regeneration or healing in general. This information is essential for a fuller understanding of future efforts in direct biologic approaches to vital pulp therapy.

(IV) *Reparative or Reactionary Dentinogenesis: Transdentinal Approach*

The ability to induce dentinogenesis in a controlled and timely manner through a remaining wall of intact dentin would be therapeutically useful. This concept of using biologic agents to control pulpal response across an existing layer of dentin, or a transdentinal approach, is a relatively new approach to a long-recognized clinical problem.

A layer of reparative dentin deep to the remaining dentin has been hypothesized to afford the pulp tissue an extra degree of protection from immediate and future external irritants (Miller and Sassler, 1962; Tronstad, 1972; Cohen and Burns, 1991). This protection is presumably provided *via* two mechanisms: First, by increasing the thickness of remaining dentin and reducing the direct connection between tubules of the primary dentin and the reparative dentin, the permeability of the dentin to transportable irritants is greatly reduced (Pashley,

1985, 1989). This reduced permeability severely limits the volume and rate of delivery of irritants (*i.e.*, dose of irritants) to the dental pulp and thus provides the pulp with additional protection. The thicker the dentin barrier and the more restrictive the connection between the cut dentin surface and the pulp *via* changes in the primary dentin tubules and the tubules in the reparative dentin, the better the barrier and thus the lower the exposure to potential irritants. Second, the added thickness of dentin increases the physical barrier between the restoration/dentin interface and underlying vital dental pulp. This extra thickness provides additional protection from subsequent thermal and mechanical challenges (Pashley, 1985, 1989). Similarly, a well-formed layer of reparative dentin deposited directly against the dentin tubules cut during cavity preparation could be instrumental in preventing subsequent pulpal disease. Since maintaining the vitality of the dental pulp tissue is a primary objective in most restorative therapies, the timely or early formation of a controlled and predictable amount of reparative dentin immediately following extensive dentin loss without overt pulp exposure would be a desirable clinical goal.

This well-formed layer of reparative dentin deposited directly against the cut dentin must be limited in its depth into the pulp and width along the primary dentin interface. An unlimited reparative dentin response has the potential of prematurely obliterating the pulp chamber, thus compromising the healing potential of the remaining pulp tissue (Torneck, 1990; Piattelli and Trisi, 1993), and reducing the chance of successful root canal therapy (Cohen and Burns, 1991; Piattelli and Trisi, 1993).

Characteristics of an Ideal Transdentinal Treatment

Reparative dentin induction should result in a controlled stimulation of a natural reparative dentin barrier along the dentin/pulp interface adjacent to the cut tubules. Ideally, the stimulant should act rapidly, function through intact cut dentin, be minimally inflammatory, and result in a limited dentinogenic response.

Materials presently available to the dental practitioner do not satisfy most of these criteria. However, soluble dentin matrix proteins and OP-1 may potentially satisfy these criteria. EDTA-soluble and collagenase-released fractions of rabbit incisor dentin extracellular matrix, but not control extracts, placed against freshly cut dentin in adult ferret canines resulted in reactionary dentin formation. The distinction between reactionary dentin as opposed to reparative dentin formation was made based on the observation that vitality of the original odontoblasts was maintained throughout the study (Smith *et al.*, 1994). Although the study did not attempt to identify which components extracted from dentin were responsible for the observed response or whether a dose-response relationship existed, it did demonstrate that pre-existing odontoblasts were capable of responding to these components. Similarly, OP-1 in solution, when applied to freshly cut dentin of non-human primate teeth, was associated with increased reactionary or reparative dentin formation in a manner dependent upon the concentration of OP-1 and the thickness of the residual dentin wall (Rutherford *et al.*, 1995). This study did not address the question of the preservation of odontoblast viability.

Future Directions

These results (Smith *et al.*, 1994; Rutherford *et al.*, 1995) provide experimental support for the hypothetical potential of biologic agents to stimulate reactionary dentin formation. Further study is needed to improve definition of the action of these agents on mature odontoblasts and their ability to satisfy the previously mentioned criteria of a transdentinal treatment. Concurrent with these studies should be the development of proper carrier vehicles that will optimize clinical handling characteristics and delivery through remaining intact dentin, which poses particularly interesting challenges because of dentin's ability to filter, buffer, and interact with agents (Pashley, 1985, 1989).

Acknowledgments

The author's work was partially supported by grants from Creative BioMolecules, Inc., Hopkinton, MA. The authors thank Paul Kaplan for the use of his diagram of OP-1.

References

Anneroth G, Bang G (1972). The effect of allogeneic demineralized dentin as a pulp capping agent in Java monkeys. *Odontol Revy* 23:315-328.

Bang G (1972). Induction of heterotopic bone formation by demineralized dentin in guinea pigs: antigenicity of the dentin matrix. *J Oral Pathol* 1:172-185.

Bang G (1973). Induction of heterotopic bone formation by demineralized dentin in rats and guinea pigs. *Scand J Dent Res* 81:230-239.

Bang G, Johannessen JV (1972). The effect of proteolytic enzymes on the induction of heterotypic bone formation by demineralized dentin in guinea pigs. *J Oral Pathol* 1:221-230.

Bang G, Urist MR (1967a). Bone induction in excavation chambers in matrix of decalcified dentin. *Arch Surg* 94:781-789.

Bang G, Urist MR (1967b). Recalcification of decalcified dentin in the living animal. *J Dent Res* 46:722-730.

Bang G, Nordenram A, Anneroth G (1972). Allogenic demineralized dentin implants in jaw defects of Java

monkeys. *Int J Oral Surg* 1:126-136.

Baume LJ (1980). The biology of pulp and dentine: a historic, terminologic, taxonomic, histological-biochemical, embryonic and clinical survey. In: The biology of pulp and dentine. Meyers HM, editor. Basel: Karger, pp. 159-182.

Bègue-Kirn C, Smith AJ, Ruch JV, Wozney JM, Purchio A, Hartmann D, *et al.* (1992). Effects of dentin proteins, transforming growth factor β_1 (TGF-β_1) and bone morphogenetic protein-2 on the differentiation of odontoblasts *in vitro*. *Int J Dev Biol* 36:491-503.

Bègue-Kirn C, Smith AJ, Loriot M, Kupferle C, Ruch JV, Lesot H (1994). Comparative analysis of TGF-βs, BMPs, IGF-1, msxs fibronectin, osteonectin, and bone sialoprotein gene expression during normal and *in vitro*-induced odontoblast differentiation. *Int J Dev Biol* 38:405-420.

Bessho K, Tanaka N, Matsumoto J, Tagawa T, Murata M (1990). Human dentin-matrix-derived bone morphogenetic protein. *J Dent Res* 70:171-175.

Bimstein E, Shoshan S (1981). Enhanced healing of tooth-pulp wounds in the dog by enriched collagen solution as a capping agent. *Arch Oral Biol* 26:97-101.

Butler WT, Mikulski A, Urist MR (1977). Non-collagenous proteins of a rat dentin possessing bone morphogenetic activity. *J Dent Res* 56:228-232.

Carmichael DJ, Dick HM, Dodd CM (1974). Histologic effects of antigenically altered collagen as a heterograft for mammalian pulp exposures. *Arch Oral Biol* 19:1121-1126.

Celeste AJ, Iannazzi JA, Taylor RC, Hewick RM, Rosen V, Wang EA (1990). Identification of transforming growth factor β family members present in bone-inductive protein purified from bovine bone. *Proc Natl Acad Sci* (USA) 87:9843-9847.

Cohen S, Burns RC (1991). Pathways of the pulp. In: Pathways of the pulp. Baltimore: Mosby Year Book Inc., pp. 447-448.

Conover MA, Urist MR (1982). Dentin matrix bone morphogenetic protein. In: Proceedings of the 1st International Conference on Chemistry and Biology of Mineralized Connective Tissue. Veis A, editor. New York: Northwestern University, pp. 597-606.

D'Souza R, Happonen RP, Ritter NM, Butler WT (1990). Temporal and spatial patterns of transforming growth factor β_1 expression in developing rat molars. *Arch Oral Biol* 35:957-965.

D'Souza RN, Letterio JJ, Dickinson DP, Roberts AB, Litz M (1995). Transforming growth factor beta and tooth development (abstract). *J Dent Res* 74:72.

Dick HM, Carmichael DJ (1980). Reconstituted antigen-poor collagen preparations as potential pulp-capping agents. *J Endodont* 6:641-644.

Finkleman RD, Mohan S, Jennings JC, Taylor AK, Jepsen S, Baylink DJ (1990). Quantitation of growth factors IGF-1, SGF/IGF-II, and TGF-β in human dentin. *J Bone Min Res* 5:717-723.

Fitzgerald M, Heys RJ (1991). A clinical and histological evaluation of conservative pulpal therapy in human teeth. *Oper Dent* 16:101-112.

Fuks AB, Michaeli Y, Sofer SB, Shoshan S (1984). Enriched collagen solution as a pulp dressing in pulpotomized teeth in monkeys. *Pediatr Dent* 6:243-247.

Fuks AB, Cleaton-Jones P, Michaeli P, Bimstein E (1991). Pulp response to collagen and glutaraldehyde in pulpotomized primary teeth of baboons. *Pediatr Dent* 13:142-150.

Glass R, Zander HA (1949). Pulp healing. *J Dent Res* 28:97-110.

Haskell EW, Stanley HR, Chellemi J, Stringfellow H (1978). Direct pulp capping treatment: a long term follow-up. *J Am Dent Assoc* 97:607-612.

Hauschka PV, Mavrokos AE, Iafrati MD, Doleman SM, Klagsbrun M (1986). Growth factors in bone matrix. *J Biol Chem* 261:12665-12674.

Heikinheimo K (1994). Stage-specific expression of decapentaplegic-Vg-related genes 2, 4, and 6 (bone morphogenetic proteins 2, 4, and 6) during human tooth morphogenesis. *J Dent Res* 73:590-597.

Heikinheimo K, Happonen RP, Miettinen PJ, Ritvos O (1993). Transforming growth factor β_2 in epithelial differentiation of developing teeth and odontogenic tumors. *J Clin Invest* 91:1019-1027.

Heine UI, Munoz EF, Flouners KC, Ellingsworth KR, Lam HYP, Thompson NL, *et al.* (1987). Role of transforming growth factor-β in development of mouse embryo. *J Cell Biol* 105:2861-2876.

Hess W (1950). The treatment of teeth with exposed healthy pulps. *Int Dent J* 1:10-14.

Huggins CB, Urist MR (1970). Dentin matrix transformation: rapid induction of alkaline phosphatase and cartilage. *Science* 167:896-897.

Inoue T, Deporter DA, Melcher AH (1986). Induction of chondrogenesis in muscle, skin, bone marrow, and periodontal ligament by demineralized dentin and bone matrix *in vivo* and *in vitro*. *J Dent Res* 65:12-22.

Jepsen S, Schlitz P, Strong DD, Scharla RH, Snead ML, Finkleman RD (1992). Transforming growth factor-β, mRNA in neonatal ovine molars visualized by *in situ* hybridization: potential role for the stratum intermedium. *Arch Oral Biol* 37:645-653.

Johannson BI, Persson I, Manera P (1963). Histologic effects of collagen and chondroitin sulphate as capping agents in amputated rat molar pulps. *Arch Oral Biol* 8:503-507.

Jones MC, Lyons KM, Hogan BLM (1991). Involvement of bone morphogenetic protein-4 (BMP-4) and Vgr-1 in morphogenesis and neurogenesis in the mouse. *Development* 111:531-542.

Joseph BK, Savage NW, Young WG, Gupta GS, Breier BH, Waters MJ (1993). Expression and regulation of insulin-like growth factor-I in the rat incisor. *Growth Factors* 8:267-275.

Jowett AK, Vainio S, Ferguson MWJ, Sharpe PT, Thesleff I (1993). Epithelial-mesenchymal interactions are required for msx1 and msx2 gene expression in the developing murine molar tooth. *Development* 111:461-470.

Kollar E (1983). Epithelial-mesenchymal interactions in the mammalian integuments. Tooth development as a model for instructive induction. In: Epithelial mesenchymal interactions in development. Sawyer RH, Fallon JF, editors. New York: Praeger Press, pp. 27-56.

Lehnert SA, Akhurst RJ (1988). Embryonic expression pattern of TGF-β type 1 RNA suggests both paracrine and autocrine mechanisms of action. *Development* 104:263-273.

Lesot H, Meyer JM, Karcher-Djuricic V, Ahmad N, Staubli A, Idriss H, *et al.*, editors (1985). Tooth morphogenesis and cytodifferentiation. Paris: INSERM.

Lesot H, Begue-Kirn C, Kubler MD, Meyer JM, Smith AJ, Cassidy N, *et al.* (1993). Experimental induction of odontoblast differentiation and stimulation during reparative processes. *Cells Mater* 3:201-217.

Lianjia Y, Yuhao G, White F (1993). Bovine bone morphogenetic protein induced dentinogenesis. *Clin Orthop Rel Res* 295:305-312.

Linde A, Goldberg M (1993). Dentinogenesis. *Crit Rev Oral Biol Med* 4:679-728.

Lyons KM, Pelton RW, Hogan BLM (1990). Organogenesis and pattern formation in the mouse: RNA distribution patterns suggest a role for bone morphogenetic protein-2A (BMP-2A). *Development* 109:833-844.

MacKenzie A, Leeming GL, Jowett AK, Ferguson MWJ, Sharpe PT (1991). The homeobox gene Hox 7.1 has specific regional and temporal expression patterns during early murine craniofacial embryogenesis, especially tooth development *in vivo* and *in vitro*. *Development* 111:269-285.

MacKenzie A, Ferguson MWJ, Sharpe PT (1992). Expression patterns of the homeobox gene, Hox-8, in the mouse embryo suggest a role in specifying tooth initiation and shape. *Development* 115:403-420.

Massagué J (1990). The transforming growth factor-β family. *Ann Rev Cell Biol* 6:597-641.

Mera K (1988). Studies on bone morphogenetic protein (BMP) derived from bovine demineralized dentin matrix. *J Jpn Stomatol Soc* 37:389-399.

Miller WA, Sassler M (1962). Permeability and staining of active and arrested lesions in dentine. *Br Dent J* 112:187-193.

Nakashima M (1989). Dentin induction by implants of autolyzed antigen-extracted allogenic (AAA) dentin on amputated dental pulps of dogs. *Endodont Dent Traumat* 5:279-286.

Nakashima M (1990). The induction of reparative dentine in the amputated dental pulp of the dog by bone morphogenetic protein. *Arch Oral Biol* 35:493-497.

Nakashima M (1994). Induction of dentin formation on canine amputated pulp by recombinant human bone morphogenetic proteins (BMP)-2 and -4. *J Dent Res* 73:1515-1522.

Orvig T (1967). Early vertebrate calcified tissues. In: Structural and chemical organization of teeth. Miles AEW, editor. London: Academic Press, pp. 45-105.

Ozkaynak E, Rueger DC, Drier EA, Corbett C, Ridge RJ, Sampath TK, *et al.* (1990). OP-1 cDNA encodes an osteogenic protein in the TGF-β family. EMBO J 9:2085-2093.

Ozkaynak E, Schnegelsberg PNJ, Jin DF, Clifford GM, Warren FD, Drier EA, *et al.* (1992). Osteogenic protein-2. *J Biol Chem* 267:25220-25227.

Pashley DH (1985). Dentin-predentin complex and its permeability: pathology and treatment overview. *J Dent Res* 64(Spec Iss):613-620.

Pashley DH (1989). Dentin permeability: theory and practice. In: Experimental endodontics. Spangberg L, editor. Boca Raton, FL: CRC Press, pp. 22-36.

Pelton RW, Dickinson ME, Moses HL, Hogan BLM (1990). *In situ* hybridization analysis of TGF-β_3 RNA expression during mouse development: comparative studies with TGF-β_1 and β_2. *Development* 110:609-620.

Piattelli A, Trisi P (1993). Pulp obliteration: a histological study. *J Endodont* 19:252-254.

Ruch JV (1985). Odontoblast differentiation and the formation of the odontoblast layer. *J Dent Res* 64:489-498.

Rutherford RB, Wahle J, Tucker M, Rueger D, Charette M (1993). Induction of reparative dentin formation in monkeys by recombinant human osteogenic protein-1. *Arch Oral Biol* 38:571-576.

Rutherford RB, Spangberg L, Tucker M, Rueger D, Charette M (1994). Time course of the induction of reparative dentin formation in monkeys by recombinant human osteogenic protein-1. *Arch Oral Biol* 39:833-838.

Rutherford RB, Spangberg L, Tucker M, Charette M (1995). Transdentinal stimulation of reparative dentin formation by osteogenic protein-1. *Arch Oral Biol* (in press).

Sampath TK, Reddi AH (1981). Dissociative extraction and reconstitution of extracellular matrix components involved in local bone differentiation. *Proc Natl Acad Sci* (USA) 78:7599-7603.

Sampath TK, Rueger DC (1994). Structure, function and orthopedic applications of osteogenic protein-1 (OP-1). *Complications in Orthop* (winter):101-107.

Sampath TK, Coughlin JE, Whetstone RM, Banach D, Corbett C, Ridge RJ, *et al.* (1990). Bovine osteogenic protein is composed of dimers of OP-1 and BMP-2A,

two members of the transforming growth factor-β superfamily. J *Biol Chem* 265:13198-13205.

Sampath TK, Maliakal JC, Hauschka PV, Jones WK, Sasak H, Tucker RF, *et al.* (1992). Recombinant human osteogenic protein-1 (h OP-1) induces new bone formation *in vivo* with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation *in vitro*. J *Biol Chem* 267:20352-20362.

Satokata I, Maas R (1994). Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nat Genet* 6:348-356.

Seltzer S (1988). Endodontology. 2nd ed. Philadelphia: Lea and Febiger.

Seltzer S, Bender IB, editors (1984). Pulp capping and pulpotomy. In: Biologic considerations in dental procedures. Philadelphia: J.B. Lippincott and Co., pp. 281-302.

Smith AJ, Tobias RS, Plant CG, Browne RM, Lesot H, Ruch JV (1990). *In vivo* morphogenetic activity of dentine matrix proteins. J *Biol Buccale* 18:123-129.

Smith AJ, Tobias RS, Cassidy N, Plant CG, Browne RM, Begue-Kirn C, *et al.* (1994). Odontoblast sitmulation in ferrets by dentine matrix components. *Arch Oral Biol* 39:13-22.

Stanley HR (1989). Pulp capping: conserving the dental pulp—can it be done? Is it worth it? *Oral Surg Oral Med Oral Pathol* 68:628-639.

Takeda K, Oida S, Goseki M, Iimura T, Maruoka Y, Amagasa T, *et al.* (1994). Expression of bone morphogenetic protein genes in the human dental pulp cells. *Bone* 15:467-470.

Thesleff I, Hurmerinta K (1981). Tissue interactions in tooth development. *Differentiation* 18:75-85.

Torneck CD (1990). The clinical significance and management of calcific pulp obliteration. *Alpha Omegan* 83:50-54.

Tronstad L (1972). Optical and microradiographic appearance of intact and worn human coronal dentin. *Arch Oral Biol* 17:847-852.

Tziafas D, Kolokuris I (1990). Inductive influences of demineralized dentine and bone matrix on pulp cells: an approach to secondary dentinogenesis. J *Dent Res* 69:75-81.

Tziafas D, Alvanou A, Kaidoglou K (1992a). Dentinogenic activity of allogenic plasma fibronectin on dog dental pulp. J *Dent Res* 71:1189-1195.

Tziafas D, Kolokuris I, Alvanou A, Kaidoglou K (1992b). Short-term dentinogenic response of dog dental pulp tissue after its induction by demineralised or native dentine or predentine. *Arch Oral Biol* 37:119-128.

Urist MR (1965). Bone: Formation by induction. *Science* 150:893-899.

Urist MR (1971). Bone histiogenesis and morphogenesis in implants of demineralized enamel and dentin. J *Oral Surg* 29:88-102.

Vaahtokari A, Vainio S, Thesleff I (1991). Associations between transforming growth factor β_1 expression and epithelial-mesenchymal interactions during tooth morphogenesis. *Development* 113:985-994.

Vainio S, Karavanova I, Jowett A, Thesleff I (1993). Identification of BMP-4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development. *Cell* 75:45-58.

Valecillos MEF, Thomas HF, Rutherford RB (1993). Immunolocalization of osteogenic protein (OP-1) in developing murine teeth (abstract). J *Dent Res* 72:110.

Veis A (1985). The role of dental pulp—thoughts on the session on pulp repair processes. J *Dent Res* 64:552-554.

Wall NA, Blessing M, Wright CVE, Hogan BLM (1993). Biosynthesis and *in vivo* localization of the decapentaplegic-Vg-related protein, DVR-6 (bone morphogenetic protein-6). J *Cell Biol* 120:493-502.

Wang EA, Rosen V, D'Alessandro JS, Bauduy M, Cordes P, Harada T, *et al.* (1990). Recombinant human bone morphogenetic protein induces bone formation. *Proc Natl Acad Sci* (USA) 87:2220-2224.

Wise GE, Fan W (1991). Immunolocalization of transforming growth factor beta in rat molars. J *Oral Pathol Med* 20:74-80.

Wozney JM (1992). The bone morphogenetic protein family and osteogenesis. *Molec Reprod Dev* 32:160-167.

Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, *et al.* (1988). Novel regulators of bone formation: molecular clones and activities. *Science* 242:1528-1534.

Yeomans JD, Urist MD (1967). Bone induction by decalcified dentin implanted into oral and muscle tissues. *Arch Oral Biol* 12:999-1008.